

Analytical

Biochemistry

An International Journal

EDITOR: *Alvin Nason*

ADVISORY BOARD:

Gary Ackers
Norman G. Anderson
Eraldo Antonini
Gilbert Ashwell
H. A. Barker
Samuel P. Bessman
Simon Black
Robert L. Bowman
Ludwig Brand
A. E. Braunstein
Robert H. Burris
Melvin Calvin
Andreas Chrambach
Frank Eisenberg, Jr.
Jacques R. Fresco
Victor Ginsburg
I. C. Gunsalus
Harlyn O. Halvorson
Osamu Hayaishi

Edward C. Heath
Leslie Hellerman
C. H. W. Hirs
Evan C. Horning
Rollin D. Hotchkiss
Walter L. Hughes
William B. Jakoby
Nathan O. Kaplan
Ephraim Katchalski
Irving Klotz
Luis F. Leloir
Oliver H. Lowry
Feodor Lynen
Brian J. McCarthy
Donald McCormick
William D. McElroy
Vincent Massey
Alan H. Mehler
Bruce Merrifield

Marshall Nirenberg
Kazuo Okunuki
Sarah Ratner
Alexander Rich
David Rodbard
Gerhard Schmidt
Michael Sela
Esmond E. Snell
Sol Spiegelman
Paul K. Stumpf
Charles A. Thomas, Jr.
Sidney Udenfriend
Bert L. Vallee
Samuel B. Weiss
Theodor Wieland
George Wolf
W. A. Wood
Lemuel D. Wright
David A. Yphantis

VOLUME 76
1976

ACADEMIC PRESS
New York and London



A Subsidiary of Harcourt Brace Jovanovich, Publishers

Copyright © 1976 by Academic Press, Inc.

All Rights Reserved

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without permission in writing from the copyright owner.

CONTENTS OF VOLUME 76

NUMBER 1, NOVEMBER 1976

RICHARD T. DEAN, LOUIS J. DEFILIPPI, AND DONALD E. HULTQUIST. Esterification of Hemins with Trimethyloxonium Tetrafluoroborate	1
MASAO HAYASHI. Interference with the Murphy-Riley Orthophosphate Determination Method in the Application to ATPase Assay	9
KATHY BECKINGHAM SMITH. A New Procedure for the Statistical Analysis of Double-Isotope Incorporation Data	16
D. B. LOWRIE. A Device for Automatic Measurement of Very Low Liquid Flow Rates	32
J. NORMAN HANSEN. Electrophoresis of Ribonucleic Acid on a Polyacrylamide Gel which Contains Disulfide Cross-Linkages	37
SUSAN S. TAYLOR, CHI-YU LEE, LESLIE SWAIN, AND PATRICK H. STAFFORD. Cyclic AMP-Dependent Protein Kinase: Purification of the Holoenzyme by Affinity Chromatography	45
K. K. ONG AND P. I. C. RENNIE. Assay Errors of Cyclic 3',5'-Nucleotide Phosphodiesterase Activity Based on the Recovery of Adenosine Alone Using an Anionic-Exchange Resin Column	53
P. B. H. O'CONNELL AND C. J. BRADY. Polyacrylamide Gels with Modified Cross-Linkages	63
B. A. BOOTH AND A. E. COLÁS. A Rapid Technique to Evaluate Two High-Affinity Binding Proteins in the Same Preparation	74
DANIEL LOUVARD, CHRISTIAN VANNIER, SUZANNE MAROUX, JEAN-MARIE PAGES, AND CLAUDE LAZDUNSKI. A Quantitative Immunochemical Technique for Evaluation of the Extent of Integration of Membrane Proteins and of Protein Conformational Changes and Homologies	83
ROBERT J. CARRICO, KWOK-KAM YEUNG, HARTMUT R. SCHROEDER, ROBERT C. BOGUSLASKI, ROBERT T. BUCKLER, AND JAMES E. CHRISTNER. Specific Protein-Binding Reactions Monitored with Ligand-ATP Conjugates and Firefly Luciferase	95
Y. H. LOO, L. SCOTTO, AND M. G. HORNING. Gas Chromatographic Determination of Aromatic Acid Metabolites of Phenylalanine in Brain	111
NEIL L. SASS AND ANTHONY R. BENEDETTO. Determination of Instantaneous Buffer Concentration for a Monotonic Flow Gradient	119
N. LING, J. LEPPÄLUOTO, AND W. VALE. Chemical, Biological, and Immunological Characterization of Mono- and Diiodothyrotropin Releasing Factor	125
HARVEY A. ITANO, KAZUHIRO HIROTA, ICHIRO KAWASAKI, AND SHIGEKI YAMADA. Mechanism and Specificity of the Phenanthrenequinone Test for Monosubstituted Guanidines	134
HARVEY S. SCHILLER. A Simple Method for Studying Steroid-Antibody Interaction under Conditions of Thermodynamic Equilibrium	142
KRISTOFFER HELLSING, HÅKAN ENSTRÖM, AND WOLFGANG RICHTER. Immunochemical Quantitation of Dextran (α -1,6-Glucan) Traces in Sucrose Solutions by a Rapid and Sensitive Automated Nephelometric Procedure	149
HENRY F. EPSTEIN AND JANICE A. WOLFF. Peptide Mapping of Contractile Proteins: Two-Dimensional Analysis of Cyanogen Bromide Fragments on Polyacrylamide Gels	157
P. D. J. WEITZMAN. Determination of Disulfide Groups in Proteins by a Sensitive Electroreductive-Colorimetric Method	170

MOSHE M. WERBER. Nucleophilicity of Isoorea Linkages in Substituted Agaroses. A Direct Method for the Determination of Alkylamino Groups Coupled to CNBr-Activated Agarose	177
ROBERT L. HEATH AND AL L. TAPPEL. A New Sensitive Assay for the Measurement of Hydroperoxides	184
PETER FELKER. A Gas-Liquid Chromatographic-Isotope Dilution Analysis of Cysteine, Histidine, and Tryptophan in Acid-Hydrolyzed Protein	192
K. LORENTZ. A Simple Polyacrylamide Gradient Gel Preparation for Estimating Molecular Weights	214
C. H. SUELTER, JEAN WANG, AND ESMOND E. SNELL. Application of a Direct Spectrophotometric Assay Employing a Chromogenic Substrate for Tryptophanase to the Determination of Pyridoxal and Pyridoxamine 5'-Phosphates	221
P. RUFFIN AND G. BISERTE. Column Chromatography of Cysteinesulfonic Acid, Cysteic Acid, and S-Sulfocysteine	233
G. M. RICHARDS. Hydrolysis with Lead Perchlorate: A New Method Used to Estimate RNA in Sugarcane Tissues	239
K. P. DUDOV, M. D. DABEVA, AND A. A. HADJIOLOV. Simple Agar-Urea-Gel Electrophoretic Fractionation of High Molecular Weight Ribonucleic Acids ..	250
ELIO F. VANIN AND ROBERT H. SYMONS. The Preparation of High Specific Activity [³ H]Chloramphenicol Base and Chloramphenicol Labeled in the Propanediol Side Chain	259
R. C. GUPTA, K. RANERATH, AND E. RANERATH. Sequence Analysis of Small Amounts of Nonradioactive Oligoribonucleotides Containing Ribose-Methylated Nucleosides by a Combination of ³ H- and ³² P-Labeling Techniques	269
HUBERT VAUDRY, CHARLES OLIVER, RICARDO USATEGUI, FRANÇOIS LEBOULENGER, MARIE CHRISTINE TROCHARD, AND RENÉ VAILLANT. Adsorption of Polypeptide Hormones to Silicate Powders: Development of Reproducible Methods for Extracting ACTH and MSH from Plasma	281
CLEMENTE LANDRISCINA, FRANCESCO M. MEGLI, AND ERNESTO QUAGLIARIELLO. A General Method for Phospholipid Polar Head Spin Labeling. Synthesis of Diphosphatidylglycerol-2-(2',2',5',5'-tetramethylpyrroline-N-oxide) Carboxylate	292
H.-R. SCHULTEN AND B. WITTMANN-LIEBOLD. High Resolution Field Desorption Mass Spectrometry V. Mixtures of Amino Acid Phenylthiohydantoins and Edman Degradation Products	300
LORIN K. JOHNSON, HOPE H. LIEBKE, AND TIMOTHY J. O'BRIEN. Fractionation of RNA Polymerase Subspecies from Soybean Hypocotyls by Isoelectric Focusing in Sephadex	311
P. H. ZASSENHAUS, K. M. HANSON, AND R. L. WOLGEMUTH. A New Water-Soluble Substrate for the Determination of Trypsin Activity	321
RICHARD W. STOUT, ROBERT J. MICHELOT, IMRE MOLNAR, CSABA HORVATH, AND JAMES K. COWARD. An Analytical Method for the Separation of Dopamine Metabolites in Cellular Extracts by High-Pressure Liquid Chromatography ..	330
RICHARD W. STOUT, CHI-DEU CHANG, AND JAMES K. COWARD. Separation of Methylated Nucleosides by High-Pressure Liquid Chromatography	342
SHORT COMMUNICATIONS	
CLAUDE COCHET, MONIQUE CHEDIN, AND EDMOND M. CHAMBAZ. Characterization of Steroid-Binding Macromolecules in Small Biological Samples by a Rapid Minigel Filtration Method	351
J. G. ROSE. The Determination of Total Blood CO ₂ and its Specific Radioactivity in 0.2-Milliliter Blood Samples	358

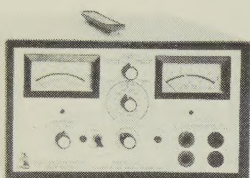
MARIA KEZDI, LADISLAU KISS, OTILIA BOJAN, TUDOR PAVEL, AND OCTAVIAN BÂRZU. 8-Bromoinosine 5'-Diphosphate, a Suitable Phosphate Acceptor of Nucleosidediphosphate Kinase	361
TETSUO HIYAMA, BERAH D. MCSWAIN, AND WILLIAM UFERT. Simultaneous Measurement of Oxygen Concentration and Absorbance Changes	365
J. W. MAYER. Simple and Rapid Methods for Drying Polyacrylamide Gels after Isoelectric Focusing	369
ALAN J. BARRETT. An Improved Color Reagent for Use in Barrett's Assay of Cathepsin B	374
A. G. HOWARD AND G. NICKLESS. The Analysis of the Betaine Homarine by High Pressure Liquid Chromatography	377
LONG-HUEI WANG, STEVEN J. MCWETHY, AND PAUL A. HARTMAN. Detection of Microorganisms That Produce Extracellular Maltases	380
HARVEY B. POLLARD AND DAN WEINGRAD. A New, Rapid Method for Measuring Multiple Samples of Cyclic AMP Using TEAE-Cellulose Column Chromatography and a Specific Binding Protein	382 •
ERRATUM	385

NUMBER 2, DECEMBER 1976

J. E. FOX AND J. M. WILKINSON. An On-Line System for Handling Data from Amino Acid and Carbohydrate Analysis	387
KEIJIRO SAMEJIMA, MASAKO KAWASE, SHUNJI SAKAMOTO, MASASHI OKADA, AND YASUO ENDO. A Sensitive Fluorometric Method for the Determination of Aliphatic Diamines and Polyamines in Biological Materials by High-Speed Liquid Chromatography	392 •
NORMAN SALEM, JR., LEO G. ABOOD, AND WAYNE HOSS. Separation of Brain Phosphatidylserines According to Degree of Unsaturation by Thin-Layer Chromatography	407
ANA PRELLER AND TITO URETA. A Hexokinase-Coupled Radioassay for Pyruvate Kinase	416
WILLIAM E. O'BRIEN. A Continuous Spectrophotometric Assay for Argininosuccinate Synthetase Based on Pyrophosphate Formation	423
THOMAS C. CURRIER AND EUGENE W. NESTER. Isolation of Covalently Closed Circular DNA of High Molecular Weight from Bacteria	431
ARTHUR KARLIN, MARK G. MCNAMEE, AND DAVID A. COWBURN. Assay of the Acetylcholine Receptor by Affinity Labeling	442
ERIC I. GRUENSTEIN AND ANDREW L. POLLARD. Double-Label Autoradiography on Polyacrylamide Gels with ^3H and ^{14}C	452
FRANCIS C. KNOWLES AND QUENTIN H. GIBSON. A Gas-Liquid Equilibration Apparatus for Quantitative Determination of Oxygen Binding by Hemoglobin ..	458
N. C. KENDRICK. Purification of Arsenazo III, a Ca^{2+} -Sensitive Dye	487
EUGENE C. BUTCHER AND OLIVER H. LOWRY. Measurement of Nanogram Quantities of Protein by Hydrolysis followed by Reaction with Orthophthalaldehyde or Determination of Glutamate	502
P. BLÜMEL AND W. UECKER. Protein Determination in the Presence of Glycerol by the Lowry Procedure. I. Influence of Reaction Temperature and Alkali	524
FRANCIS E. DWULET AND FRANK R. N. GURD. A Comparison of Sulfonated Phenylisothiocyanates for Reducing Losses of Lysine-Containing Peptides during Automated Sequencing	530
PETER J. HOFFMANN AND ROGER CHALKLEY. A Neutral pH Acrylamide Gel Electrophoretic System for Histones and Other Basic Proteins	539

RAGNAR FLENGSRUD. Detection of Amino-Terminal Tryptophan in Peptides and Proteins Using Dansyl Chloride	547
A. PRESCOTT AND D. MANGNALL. Interference by Sucrose in the Chemical Determination of Citrulline with Diacetyl Monoxime	551
H.-M. VAN DE LOO. An Improved Method for the Quantitative Determination of Hexosamines According to Elson and Morgan	556
NORMAN HALL AND MARLENE DELUCA. Electrophoretic Separation and Quantitation of Creatine Kinase Isozymes	561
JAMES C. READING AND HARRISON M. LAZARUS. Statistical Analysis of Molecular Weight Distribution of Macromolecules	568
RICHARD LEVENSON. A Collagen Film Microassay for Tissue Collagenase	579
CHRISTOPHER M. WEST AND DANIEL MCMAHON. A Physical Explanation for Multiple-Cell Classes after Centrifugation in Colloidal Silica Gradients	589
K. GREEN AND S. STEFFENRUD. Method for Quantitative Analysis of PGA_2 from Plasma Using Deuterated Carrier and Gas-Liquid Chromatography-Mass Spectrometry	606
S. P. MAHADIK, A. KORENOVSKY, AND M. M. RAPPORT. Slab Gel Analysis of the Polypeptide Components of Rat Brain Subcellular Organelles	615
HADASSAH TAMIR, SAHEBARAO P. MAHADIK, AND MAURICE M. RAPPORT. Fractionation of Synaptic Membranes with Sodium Diatrizoate	634
SHORT COMMUNICATIONS	
PAUL W. CHUN, SHIH Y. SHIAO, EUGENE E. SAFFEN, DINESH O. SHAH, W. JAPE TAYLOR, AND RICHARD J. DiTORE. Measurements of the Apparent Interfacial Viscosity of the Red Blood Cell	648
COLLIN R. GARDNER, ROBERT S. LANGER, AND CLARK K. COLTON. Dependence on pH of the Hydroxylamine Assay for Acyl Phosphates	654
ERRATUM	657
AUTHOR INDEX FOR VOLUME 76	659
CUMULATIVE SUBJECT INDEX FOR VOLUMES 70-76	662

Instruments for Electrophoresis from ISCO



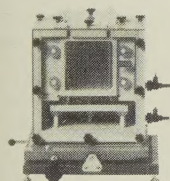
Power supplies

Pulseless constant power (wattage) prevents overheating due to variations in systems resistance, while still maintaining the highest possible separation speed. Superior line and load regulation suits the Model 493 for precise analytical electrophoresis; 200 watt capacity accommodates the largest preparative apparatus.

Preparative Gel Electrophoresis Apparatus

Colora UltraPhor®

The exclusive dual buffer elution system of this large slab apparatus produces a greater sample yield and one-tenth the dilution common in other apparatus. Developing preparative procedures is greatly simplified as they may be derived from analytical separations performed on the same equipment.



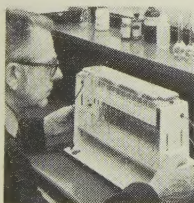
By combining the separating power of gels with the zone storage and retrieval convenience of density gradients, the ISCO ELECTROSTAC® separator provides the least dilution of any available micro-preparative electrophoresis technique.

ELECTROSTAC®



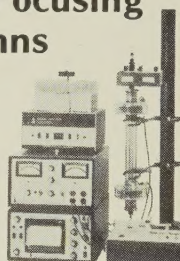
Analytical Gel Electrophoresis Apparatus

Suitable for both electrophoresing gels and destaining, the Model 1270 features a linear alignment of gel tubes and a bottom tank which can be easily lowered for access to all the tubes. Unlike circular units, this configuration does not require removal and emptying of the upper tank when changing tubes.



Density Gradient and Isoelectric Focusing Columns

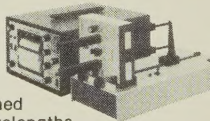
ISCO density gradient electrophoresis columns provide the researcher with the means of periodically scanning zone separation during electrophoresis and precisely recovering zones with minimal loss of resolution.



Gel Scanner

Gels electrophoresed in quartz tubes can be scanned at UV wavelengths during separation, and after electrophoresis they may be stained and scanned at visible wavelengths.

A gel may simultaneously be scanned with a blank, remove background ampholyte absorbance in isoelectrically focused gels.



Literature

Applications research bulletins describing ISCO instruments are yours for the asking. For a look at the entire line of biochemical research instruments, send for the new 64-page catalog; and if you'd like a handy booklet hard-to-find data and tables, ask for a free copy ISCOTABLES.



ISCO

Box 5347

Lincoln, Nebraska 68505

Phone (402) 464-0231

NEW MILES FROM RESEARCH PRODUCTS

MANUFACTURERS OF THE PENTEX® BRAND OF BLOOD PROTEINS FROM VARIOUS SPECIES

FIBRINOGENS, (FRACTION I)

For use in procedures for determining Thrombin and other clotting factors by kinetic and/or clot opacity techniques.

GAMMA GLOBULINS, (FRACTION II)

For co-precipitation in radio-immunoassay methods where antigen-antibody complex is separated from free antigen by protein precipitation techniques.

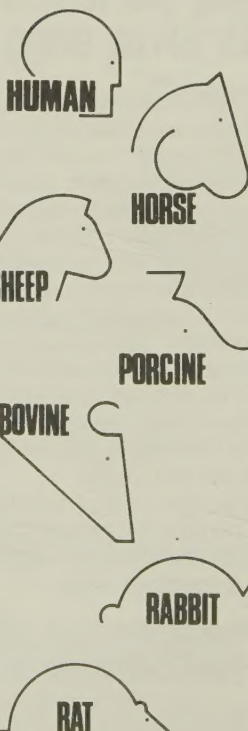
For immunological studies involving tolerances, association kinetics, transport and binding research, antigen-antibody response, antibody synthesis, etc.

BETA GLOBULINS (FRACTION III)

The source of the various grades of Thrombin for use in clotting factor assays and studies or in routine coagulation tests.

ALPHA GLOBULINS (FRACTION IV)

For growth promoting activity and/or inhibition studies and as a source of Thyroxine binding globulin for purification or for affinity binding of Thyroxine.



ALBUMINS (FRACTION V) CRYSTALLIZED

Powders and solutions for use in studies involving binding, dilution, stabilization and transport.

FRACTION V

In various forms, grades, derivatives and sizes utilized in transport, growth promotion, activation, enhancement, supplementation and stabilization techniques.

MONOMERIC

Bovine powder and solution as a reference standard for colorimetric assay methods, protein analytical techniques or as a molecular weight standard in physical studies.

REAGENT SOLUTIONS

In varying concentrations with and without preservatives and additives for use in immunohematological procedures, dilution, standardization, growth promotion and density gradient separation techniques.



Research Products
Miles Laboratories, Inc.
Elkhart, Indiana 46514
Phone: 219-264-8804

More detailed description of these products can be found in our new Biochemicals Catalog. If you don't have one, write the office nearest you for your free copy.

Academic Press publishes books and journals in many areas of the biological, medical, and biomedical sciences including:

anatomy, histology and cell biology
biochemistry and molecular biology
cancer research and oncology
cardiology and the vascular system
environmental science
food science and nutrition
genetics and human development
immunology and hematology
microbiology and virology
neurosciences, neurology and psychiatry
oceanography and marine biology
ophthalmology and otolaryngology
pathology, clinical pathology and parasitology
pharmacology, therapeutics and toxicology
physiology, biophysics and biostatistics
radiology and nuclear medicine
reproductive and perinatal medicine

For a list of titles in your subject area,
please write to the publisher, attention:
Sales Department.

AP 7400

ACADEMIC PRESS

A Subsidiary of Harcourt Brace Jovanovich, Publishers

111 FIFTH AVENUE, NEW YORK, N.Y. 10003

24-28 OVAL ROAD, LONDON NW1 7DX